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⑤④ **Elastase-containing hair care agent.**

⑤⑦ Elastase is contained as an effective ingredient in a hair care agent such as hair ointment or lotion. The hair care agent of this invention is effective in promoting the growth of hair.

**EP 0 166 337 A1**

This invention relates to a hair care agent containing elastase as an effective ingredient. Elastase itself has already been known. Therefore, this invention is directed to new use of elastase for the purpose of hair care in the fields of pharmaceuticals and cosmetics. The term "hair care" as used herein must be interpreted in broad sense, namely, it means to invigorate, restore, refresh and/or stimulate the hair. The subject matter of this invention, hair care agent, must hence be distinguished from mere hair dressings which are routinely used in grooming and styling the hair.

Elastase is an enzyme which serves to singularly degrade elastin, a water-insoluble scleroprotein. Biochemically, it has been recognized to have  $\beta$ -lipoproteinase activity and lipoproteinlipase activity and acts to normalize lipid dysbolism in both serum and tissues. It is thus clinically used to improve serolipid disorder which is induced along with hyperlipemia and arteriosclerosis. It also acts for arterial walls per se to maintain and enhance their elasticity and distensibility. In other words, it has such functions

that modified elastin is taken away from arterial walls, production of fresh elastin is promoted, deposition of fat on modified elastin is suppressed, and development of atherosclerosis is inhibited. Reference may be made to the following articles 1) - 6), which are written in the Japanese language except for the article 6).

- 1) OGAWA, Kazuro; and WAGASATO, Yasuhiro:  
"Morphological Study on the Anti-athero-  
sclerosis Effect of Elastase", Nichiroishi,  
10, 277-292 (1973);
- 2) OSAWA, Akira; "Antiartherosclerotic Effect of  
Elastase (Elastolytic Enzyme)",  
Nichinaikaishi, 59, 20-29 (1970);
- 3) NAITO, Shuko; TONO, Toshio; IWABUCHI,  
Tsutomu; ISHIMARU, Yoshie; UESUGI  
Masahide; OGASAWARA, Michio; OMORI, Akimasa;  
KASE, Masao; KIMURA, Hitoshi; NANASATO,  
Yasushi; and YOKOYAMA, Saburo: "Discussion by  
the Double Blind Test on the Effects of  
Elastase for Improving Serolipid Disorder",  
Igaku no Ayumi, 82, 848-859 (1972);
- 4) TONO, Toshio: "Treatment of Arteriosclerotic  
Diseases by Elastase", Rinsho to Kenkyu,  
53, 1798 (1976);
- 5) HASEGAWA, Mctoharu; KAWASAKI, Ken; ARAI,

Chikao et al: "Antiartherosclerotic  
Activities of Elastase", Domyaku Koka,  
18(2), 271-286 (1980); and

- 5 6) Balo, J. and Banga, I.: "The Elastolytic  
Activity of Pancreatic Extracts", Biochem.  
J., 46, 384 (1950).

In addition to the above-mentioned application  
of elastase for hyperlipemia, arteriosclerosis and  
hypertension, it has been found that elastase can also  
10 be used effectively for diabetes and renal diseases  
developed along with diabetes, resulting in the  
development of new utility for elastase. In this  
concern, reference may be made to the following  
articles 7) - 9), the first two of which are written in  
15 the Japanese language.

- 7) OIKAWA, Shinichi; KAKIZAKI, Masae; and  
GOTO, Yoshio: "Inhibition of Tylosis of  
Basilemma of Rat Renal Glomeruli, Caused by  
Spontaneous Diabetes, by Pancreatic  
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(1979);

- 8) KASEDA, Nobuhiro: "Effects of Elastase on  
Initial Renal Lesion of KK Mice Suffering  
from Spontaneous Diabetes - Morphological  
25 Observation by Electron Microscope", Rinsho  
Seijinbyo, 7, 133-139 (1977); and

- 9) SHIBATA, Masao; KASAI, Atsuko; KISHI,  
Tsunenori; KOBAYASHI, Kaizo; YASUDA, Bunji;  
KUNO, Jyoji; and SASAKI, Minoru: "Effects of  
Elastase on Glomerular Lesion of N.S.Y. Mice  
Suffering from Spontaneous Diabetes", Nagoya  
J. Med. Sci., 43, 111 (1981).

One of the present inventors conducted a variety  
of investigations with a view toward developing new  
pharmaceutical utility on elastase. As a result, it  
was found that elastase is effective for the removal of  
lipid accumulated in histiocytic cells and in addition,  
can significantly activate aged vascular smooth  
muscles. Reference may be made to the following  
article 10) which is written in the Japanese language.

- 10) MATO, Masao; OKAWARA, Shigeo; SANO,  
Motohiko; and KURIHASHI, Kyoichi: "Study on  
Cleaning Cells in Brain (Fluorescent Granular  
Circumferential Cells) - Especially,  
Incorporation of Hemolipid", No to Shnkei,  
34, No.10, 989 - 997 (1982).

One of the present inventors proceeded  
with a further investigation. As a result, it came to  
his attention that elastase shows inhibitory effects

against depilation which is developed as a side effect upon administration of adriamycin, an anticarcinomelcositic agent. In an experiment on aged animals, it was also observed that an elastase-applied group  
5 generally had fine coats of hair and developed less effluvium capillorum. Such findings were unexpected findings which were not inferable or conceivable from the previous findings on elastase. Further experiments have then been carried out on the basis of such  
10 findings, leading to completion of this invention.

As apparent from the foregoing, an object of this invention is to provide a hair care agent containing elastase as an effective ingredient.

In one aspect of this invention, there is thus  
15 provided a hair care agent containing elastase as an effective ingredient.

The hair care agent of this invention is effective in promoting the growth of hair.

The above and other objects, features and  
20 advantages of this invention will become apparent from the following description and the appended claims, taken in conjunction with the accompanying drawings.

25 Figs. 1 and 2 are histograms each of which shows the distribution in length of hairs on the backs of

rats tested in Experiment 1, which will be described herein.

5            Industrially, elastase is extracted and provided using swine pancreas. As elastase useful in the practice of this invention, it is possible to employ not only elastase obtained by such an extraction technique but also that produced by synthetic or  
10       fermentation techniques. No special limitation should thus be imposed on elastase in terms of its origin or preparation technique. Certain characteristic features of elastase extracted and provided using swine pancreas as a raw material will be given next by way of example.  
15            First of all, its molecular weight, isoelectric point, sedimentation coefficient and  $w$  are 25,900 (a value determined from the one-dimensional arrangement of amino acids),  $\text{pH } 9.5 \pm 0.5$ ,  $S_{20}$  and 2.6, respectively. Turning next to its activities, it  
20       contains serine and histidine at its active sites and has such properties that in addition to elastin, it degrades singularly and significantly the methyl ester of N- $\alpha$ -benzoyl-L-alanine or acetyl-L-trialanine p-nitroanilide which is a synthetic substrate.  
25       According to measurement results obtained using the methyl ester of N- $\alpha$ -benzoyl-L-alanine as a substrate,

the optimum pH for its activities ranges from 8 to 10 with 8.8 or so being particularly preferred. On the other hand, its activities are inhibited by NaCl, KCl,  $(\text{NH}_4)_2\text{SO}_4$ , NaCN and  $\text{CuSO}_4$ . Its activities are also inhibited by certain N- $\alpha$ -benzoylcarboxyl derivatives.

As defined at the beginning of this specification, the term "hair care" as used herein has a definition which should be interpreted in broad sense. As will become apparent from Experiment 1 which will be given herein, the term "hair care" means to promote the outgrowth of new hairs and to foster outgrown hairs and at the same time, embraces stimulation of scalp metabolism and hence prevention of depilation. Hairs are formed by hair bulb cells. After their growth over a certain period of time, they enter the degeneration stage and will eventually be subjected to depilation. During this hair life cycle, elastase have such effects as stimulating the outgrowth of new hairs, promoting the growth of outgrown hairs, and inhibiting their prematured degeneration and depilation. Elastase has such hair care effects as defined by the combination of these effects. Therefore, the term "hair care" as used herein must be interpreted in the above-described concept.



Application of the hair care agent of this invention is effected principally by coating it directly to each intended site on the scalp. It has been found empirically that the hair care agent is also  
5 effective by oral or rectal administration instead of its coating application. Therefore, the present invention shall not be limited to any particular application method. The hair care agent of this invention may however be formed into preparations  
10 suitable for its coating application in a principal embodiment of this invention, because it is most preferred to apply the hair care agent by its coating in order to draw out its effects promptly. The amount of elastase to be used and its toxicity will vary  
15 depending on the manner of its application. However, they may generally be as follows. When coated, the amount of elastase cannot be limited specifically because it varies considerably depending on the frequency of application and the density of hairs per  
20 unit area. It may instead be possible to define the concentration of elastase in the hair care agent of this invention. Its preferred concentration may be 0.05 - 0.2 w/v %. It should however be borne in mind that the present invention is by no means limited by or  
25 to this concentration range. When coated, no particular toxicity against the scalp was observed.

When administered orally, the dose of elastase may for example be 2,000 - 20,000 EL.U. (elastase unit) per day. It is preferred to administer elastase at the above dose level, for example, for 4 - 20 weeks continuously. It should however be borne in mind that the present invention is not limited by or to this dose range.

Elastase has such toxicity as summarized in the following table.

10 Acute Toxicity LD<sub>50</sub> (EL.U./kg)

Animal	Sex	Oral	Sub-cutaneous	Intraperitoneal	Intravenous
Rat	♂	>150,000	>75,000	6,380	6,380
	♀	>150,000	>75,000	5,850	6,380
15 Mouse	♂	>150,000	>75,000	4,970	5,100
	♀	>150,000	>75,000	2,780	4,310

Other toxicity will be shown in the following various experiments.

Subacute toxicity:

20 Elastase was orally administered at 750, 7,500, 37,500 and 75,000 EL.U./kg/day to male and female Wistar rats for straight 4 weeks and at 900 and 4,500 EL.U./kg/day to male and female beagles for straight 12 weeks.

25 As a result, no abnormal or pathogenic finding which is worthy to mention specifically was recognized

in general appearance, blood and urine tests and morphological observation (both visual and histological).

Chronic toxicity:

5           Elastase was orally administered at 2,250, 5,700, 11,250 and 22,500 EL.U./kg/day to male and female Wistar rats for straight 24 weeks.

As a result, no abnormal or pathogenic finding which is worthy to mention specifically was recognized  
10 in general appearance, blood and urine tests and morphological observation (both visual and histological).

Teratogenicity:

In the organofaction stages of pregnant mice and  
15 rats, elastase was forcedly and orally administered at 750, 7,500 and 75,000 EL.U./kg/day to them for straight 6 days. As a result, there were observed neither death, growth suppression and teratogenic effects on their fetuses nor effects on morphological and  
20 functional differentiation of the neonates.

When the hair care agent of this invention is applied to the scalp, it is preferred to form it into a liquid lotion as a specific preparation form thereof. However, no problem or inconvenience will arise when it  
25 is formulated into a cream-like ointment. It should thus be born in mind that the present invention is not

limited by or to any specific preparation form.

Although elastase is an essential ingredient in the hair care agent of this invention, it is free to choose and incorporate, as ingredients for the hair care agent of this invention, fat-soluble vitamins such as vitamin E, nicotinic acid and vitamin E, antihistaminic agents, hormones, vasodilators and/or the like. It should however be borne in mind that the present invention is not limited specifically by or to the incorporation of these optional ingredients. Each preparation form suitable for application to the scalp may be obtained by a method known per se in the art, using ingredients, required for the preparation of the corresponding preparation form, as materials. When it is desired to orally administer the hair care agent of this invention, it may be formed into one or more preparation forms suitable for oral administration, for example, into granules, tablets, capsules and so on. Each of these preparation forms may be obtained in a manner commonly known in the art, using a usual vehicle or excipient which is routinely employed in the technical field of preparation forms.

The present invention will be described specifically by the following Examples.

## Example 1:

Silicone oil (30 g), polyoxyethylene-hardened  
castor oil (0.5 g) and a phosphate buffer of pH 7 (in a  
amount required to make the total volume to 1 liter)  
5 were added to 95% ethyl alcohol (950 ml). After  
dissolving the mixture into an intimate solution at  
ordinary temperature, elastase (2 g) was added with  
stirring to prepare a uniformly-suspended lotion as a  
scalp-coating hair care agent of this invention. It  
10 was filled in sealed containers and stored below 30°C.  
It was necessary to shake the containers prior to each  
application.

## Example 2:

Cholesterol (5 g), purified soybean lecithine (2  
15 g), glycerin (30 g) and silicone oil (10 g) were added  
to 95% ethyl alcohol to make the total volume to 1  
liter. After dissolving the former to form a  
homogeneous clear solution, elastase (1 g) was added  
with stirring to prepare a uniformly-suspended lotion  
20 as a scalp-coating hair care agent of this invention.  
It was filled in sealed containers and stored below  
30°C. It was necessary to shake the containers prior  
to each application.

## Example 3:

25 Butoxypolyalkyleneglycol ether (150 g), Vancide  
(trade mark) (1 g), polyoxyethyleneoleyl alcohol ether  
(15 g) and perfume (15 g) were uniformly dissolved in

95% ethyl alcohol (800 ml),, followed by addition of propylene glycol (30 g) and a phosphate buffer of pH 6.5 (in an amount required to make the total volume to 1 liter) to obtain a clear solution. Elastase (1 g) was added with stirring to the solution to prepare a uniformly-suspended lotion as a scalp-coating hair care agent of this invention. It was filled in sealed containers and stored below 30°C. It was necessary to shake the containers prior to each application.

10 Example 4:

Elastase (85 EL.U./mg) (100 g) and the sucrose ester of a fatty acid (400 g) were ground gently into uniform powder, in which spray-dried lactose (500 g), crystalline cellulose (495 g) and CMC calcium (300 g) were mixed. Thereafter, calcium stearate (5 g) was sprinkled through a 80-mesh sieve. The resulting mixture was mixed uniformly and punched into tables, each of 8 mm across and 180 mg heavy, thereby forming a hair care agent of this invention suitable for oral administration.

Effects of this invention will next be described by the following Experiment:

Experiment 1:

Text materials:

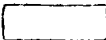
25 Provided as a test sample was a lotion prepared in accordance with the procedure described in Example 2. In addition, another lotion which had been prepared


in the same manner as in Example 2 except for the omission of elastase was provided as a control.

Method :

As laboratory animals, Wistar rat groups ( $\hat{\phi}$ ) of 5 months old and Wister rat groups ( $\hat{\phi}$ ) of 50 months old were used. Back hairs of each rat was removed by a depilatory and its back was washed thoroughly. Then, the test sample and control were coated everyday in an amount of 0.5 - 1 ml per 10 cm<sup>2</sup> of the skin to its left half area and its right half area respectively. To the rat groups of 5 months old, the test sample and control were coated everyday for 20 days. On the other hand, they were coated everyday for 45 days to the rat groups of 50 months old. Hairs were then pulled out from both areas. Out of these hairs, the lengths of 100 hairs were measured. Measurement data obtained by repeating the above procedure twice or more were averaged.

Results:

Results are shown in Figs. 1 and 2. Namely, Figs. 1 and 2 are histograms showing the distribution in length of hairs on the backs of the rats under the experimental conditions. Hair lengths (mm) are plotted along the axis of abscissas, while the number of hairs in each length range is plotted along the axis of ordinates. In the drawings, plain rectangles  indicate results on the control while hatched

rectangles  designates results on the test sample. Fig. 1 summarizes results obtained on the rat groups of 50 months old, while Fig. 2 summarizes results collected on the rat groups of 5 months old.

5 It is clearly envisaged from Fig. 1, which pertains to the aged rats having extremely declined hair growth, that the hair care agent of this invention is effective in promoting the growth of such hairs by as much as about twice. In the case of the young rats featuring  
10 fast hair growth, it is understood from Fig. 2 that the hair care agent of this invention is also effective in promoting the growth of hair.

Having now fully described the invention, it will be apparent to one of ordinary skill in the art  
15 that many changes and modifications can be made thereto without departing from the spirit or scope of the invention as set forth herein.



CLAIMS:

1. A hair care agent comprising elastase as an effective ingredient.

2. A hair care agent as claimed in Claim 1,  
5 wherein the hair care agent is in a preparation form suitable for its application to the skin.

3. A hair care agent as claimed in Claim 2,  
wherein the content of elastase is 0.001 - 1.0 w/v %.

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Fig. 1

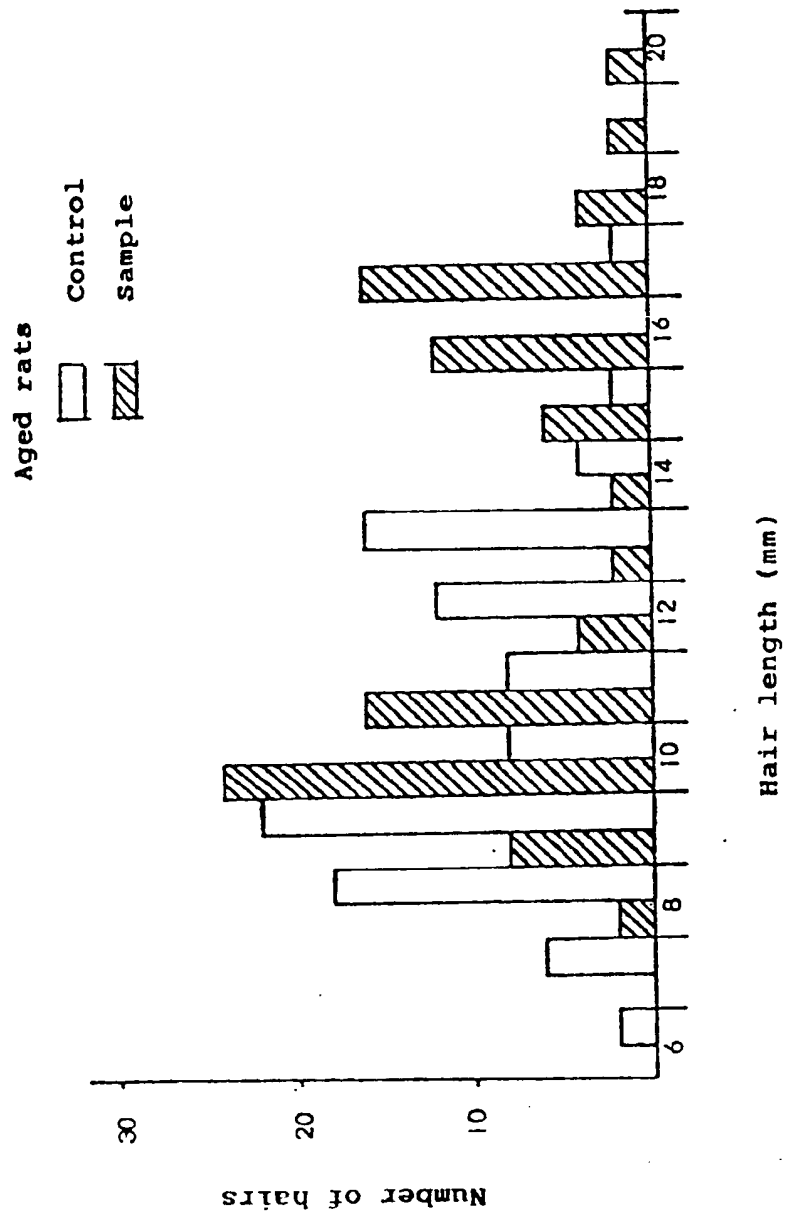
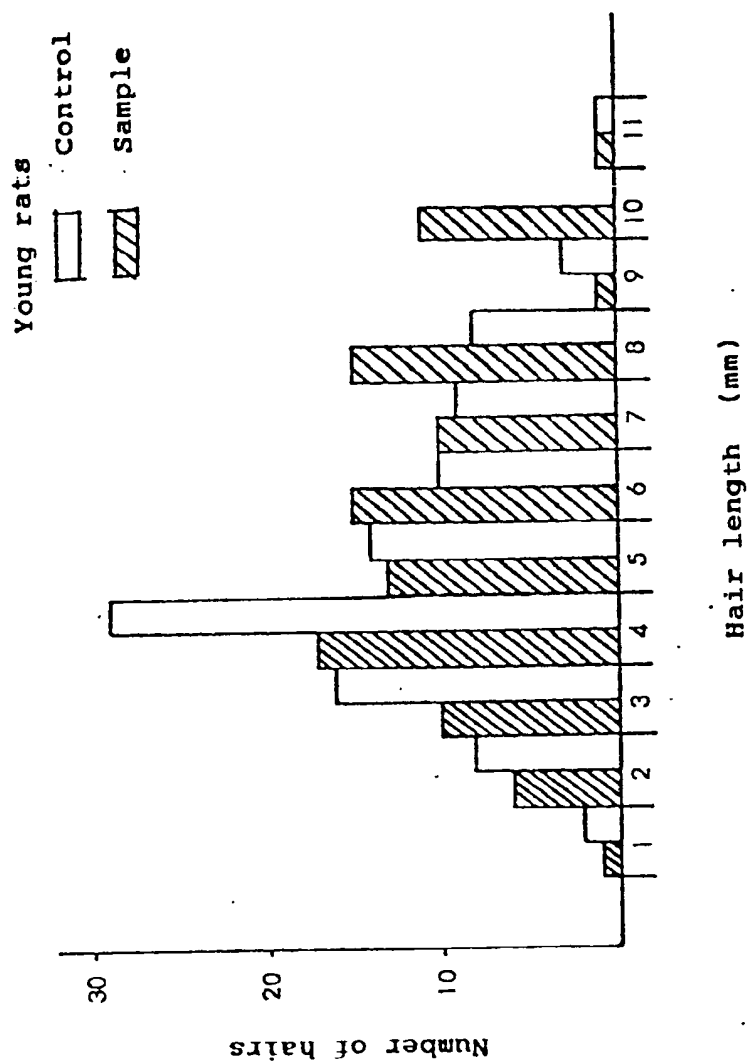


Fig. 2





European Patent  
Office

# EUROPEAN SEARCH REPORT

0166337  
Application number

EP 85 10 7430

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl. 4)
X	FR-A-1 523 250 (LABORATOIRES ALBERT ROLLAND) * Abstract *	1-3	A 61 K 7/06
X	--- CHEMICAL ABSTRACTS, vol. 93, 1980, page 318, no. 31635k, Columbus, Ohio, US; & SU - A - 719 630 (ALL-UNION SCIENTIFIC-RESEARCH BIOTECHNICAL INSTITUTE; "FLORA" INDUSTRIAL ENTERPRISES OF CONSUMER CHEMISTRY) 05-03-1980	1-3	
X	--- EP-A-0 097 810 (RAVIZZA S.P.A.) * Claims 1,5 *	1	
A	--- GB-A-1 448 276 (EISAI CO.) * Claims; page 1, lines 28-40 *	1	TECHNICAL FIELDS SEARCHED (Int. Cl. 4)
			A 61 K
The present search report has been drawn up for all claims			
Place of search THE HAGUE		Date of completion of the search 05-09-1985	Examiner WILLEKENS G.E.J.
CATEGORY OF CITED DOCUMENTS			
X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document	